Inhibition of Ocular Herpes Simplex Infection in Rabbits by Extracts of Burkitt's Lymphoma Cell Cultures[1] (35841)

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An inhibitor of herpes simplex virus (HSV) has been found in sonicated extracts of cell pellets of a lymphoid cell line derived from a Burkitt's lymphoma (1). The inhibitor was demonstrable in monolayer cultures of rat and human kidney cells in both plaque inhibition and 24-hr growth inhibition experiments. Although it was active against HSV, it did not significantly inhibit vesicular stomatitis virus (VSV) in similar experiments. The inhibitory activity was destroyed by boiling for 5 min, and exposure to pH 2 for 16 hr at 4°, and it was decreased fourfold by incubation with 2 mg/ml of trypsin for 1 hr at 37°. Since the lymphoma cell line produced immunoglobulins *in vitro*, the possibility that the inhibitor was an antibody to HSV was considered; however, there was no evidence of virus inactivation in a neutralization test in which virus was preincubated with the extract. On the basis of its biological properties, it was thought that the inhibitor was not interferon or an interferon inducer, and the possibility that it was some type of repressor involved in the maintenance of the EB herpesvirus in a latent state in the lymphoma cells was considered.

The present studies were undertaken to determine if this inhibitor would be effective *in vivo* in the treatment of herpes simplex disease. The specific model used was herpes simplex keratitis in rabbits and the Burkitt's lymphoma extract was instilled topically into eyes infected with HSV.

Materials and Methods. The extract used was derived from AL-1 cultures of Burkitt's lymphoma, a cell line originating from a jaw tumor of a Nigerian boy (2). Cell suspensions containing 10^8 AL-1 cells/ml were obtained by low speed centrifugation and frozen at −20°. When suitable volumes had been collected, they were thawed and 10-ml samples were placed in a 50-ml beaker and sonicated at maximum amperage with a Branson probe sonicator for 1 min. The sonicated material was then centrifuged in a Beckman Model L ultracentrifuge with a No. 40 head for 2 hr at 30,000 rpm and the supernatant was frozen and stored in liquid nitrogen. An extract was similarly prepared from HEp-2 human epidermoid carcinoma cells (10^8 cells/ml) for use as a control.

An oral strain of HSV (No. 11124) was used to infect the corneas. The virus pool was prepared in roller bottles of HEp-2 cells and contained $10^{7.07}$ pfu/ml in a plaque assay with a methyl cellulose overlay on LBN rat kidney cells as previously described (3). Male albino New Zealand rabbits weighing 1.5 to 2 kg were used as the experimental animals. The rabbits were anesthetized with intravenous Nembutal and the right corneas were scratched with a 20-gauge needle to produce two horizontal and two vertical lines extending from limbus to limbus, each approximately 0.3 mm in depth. One-tenth ml of a suspension containing HSV (10^6 pfu) was dropped into the lower cul-de-sac of the right eye, following which the lids were manually closed and rubbed against the eye for 30 sec.

Experiments and Results. Two experiments were carried out. In the first experiment, 48 hr after the right cornea of 20 animals had been abraded and infected, the animals were divided into 10 pairs, each pair having similar appearing lesions. One animal of each pair was treated with Burkitt's lymphoma extract and the other animal served as a control. Four times daily 2 drops (approx 0.1 ml) of

[1] This work was supported by U.S. Public Health Service grant EY-00108-02.

the Burkitt's lymphoma extract diluted 1:2 with tissue culture media (RPMI 1640 containing 20% fetal calf serum) was instilled into the lower cul-de-sac of the right eye of each treated animal and the lids were held closed for 30 sec. Treatment was continued for 7 days. Each control animal received the tissue culture media alone in a similar manner to the infected eye.

In the second experiment, treatment with Burkitt's lymphoma extract was started at 24 hr after corneal abrasion and virus infection. In addition to the control and experimental groups used in Exp. 1, there were 2 additional groups: (i) animals treated with an AL-1 extract in a dilution of 1:5; (ii) a group receiving a 1:2 dilution of an extract of HEp-2 cells that had been prepared in a manner identical to that of the Burkitt's lymphoma extract. There were 6 animals in each group. Two drops of the respective solutions were instilled into the lower cul-de-sac 4 times daily for 7 days.

Both eyes of every rabbit were examined daily with a magnifying loupe and hand light, and a slit-lamp biomicroscope both before and after the corneas were stained with fluorescein. The severity of keratoconjunctivitis thus observed was graded on a scale of 0 to 4: Grade 0 was an essentially normal appearing eye. Grade 1 constituted a well-defined epithelial lesion with slight superficial edema of the stroma limited to an area underlying the epithelial lesion; or punctate staining of the cornea. Grade 2 consisted of a more diffuse epithelial ulcer with moderate stromal edema extending beyond the epithelial defect. Grade 3 was an epithelial defect involving less than half of the corneal epithelium with severe stromal edema. Grade 4 described a defect involving more than half of the corneal epithelium with the stroma having a dense white appearance suggesting necrosis. At times ranging from 8 to 21 days after inoculation, the animals were sacrificed; and the eyes were removed and fixed in 10% buffered formalin for histopathologic study. In addition, the brains of animals with signs of encephalitis were sectioned and studied microscopically.

In the first experiment, both the treated and control groups showed an initial worsening of the keratoconjunctivitis (Fig. 1). By the fourth day of treatment, improvement was noted in the treated group while the control group continued to do poorly. Two control animals died with signs of encephalitis during the first 9 days; no treated animals were so affected.

The clinical results for the second experiment are shown in Fig. 2. Two of the control animals treated with media alone and one of the control animals receiving HEp-2 extract developed signs of encephalitis and died dur-

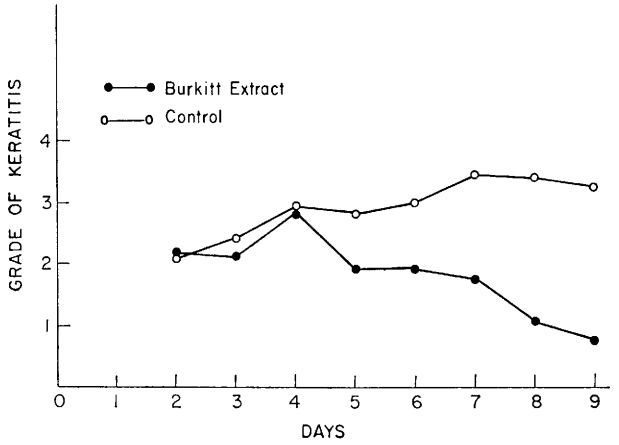

FIG. 1. Response of herpetic keratoconjunctivitis to topical treatment with Burkitt's lymphoma extract. Treatment was started 48 hr after the cornea was abraded and HSV was instilled. The control group was treated with tissue culture media alone. Grades represent the mean of 10 animals/group.

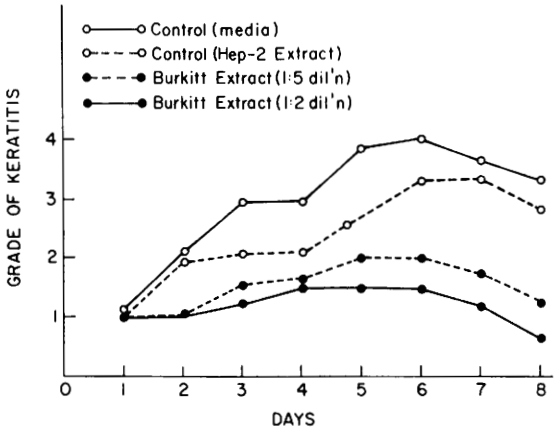

FIG. 2. Response of herpetic keratoconjunctivitis to treatment with Burkitt's lymphoma cell extract. Treatment started 24 hr after corneal abrasion and instillation of herpes simplex virus. The concentrations used and types of control media are indicated on the graphs. Grades represent mean of 6 animals/group.

ing the period of treatment.

Histopathologic studies of the eyes of animals treated with the Burkitt's lymphoma extract showed generally less severe or absent keratitis, uveitis, retinitis, and papillitis in the experimental eyes as compared to the control animals. Papillitis was seen in the noninfected (left) eye of the untreated animals but the optic nerve was normal in the noninfected eyes of most of the treated animals. Areas of necrosis with lymphocyte infiltration and foci of glial and vascular-endothelial proliferation were noted in the brains of animals dying of encephalitis.

Discussion. Herpetic keratitis is a major problem in clinical medicine and is the most frequent corneal infection recognizable on an etiologic or morphologic basis. The incidence and severity of the disease has been increasing and this fact has been correlated with the increasing use of topical corticosteroid hormones in the eye. Discovery by Kaufman (4) that 5-iodo-2′-deoxyuridine cures or ameliorates herpes simplex keratitis has been an important advance in therapy. The response to treatment in many cases, however, remains unsatisfactory. The therapeutic use of interferon inducers such as polyI-polyC is an interesting possibility now being studied by a number of investigators (5). A soluble protein fraction has been described in the lysate of *Escherichia coli* infected by lambda-phage which inhibits intracellular replication of herpes simplex and vaccinia viruses (6, 7). Experimentally induced herpetic ulcers in the corneas of rabbits treated with this material were described as being of smaller size than those in control animals, but no clinical trials with this material have been reported.

Loss of vision due to ocular herpes infections is related to the chronic and recurrent nature of this infection. Several explanations have been offered to explain the long intervals that often occur between clinical attacks and the source of reinfection: (i) chronic asymptomatic infection of the conjunctiva and lacrimal gland has been demonstrated which could provide a source for recurring corneal infection (8); (ii) small foci of active infection have been demonstrated in corneal tissue removed during transplantation which might give rise to subsequent widespread infection (9); (iii) it has been hypothesized that herpesvirus lies dormant and undetectable in the cells of the involved tissue and becomes reactivated from this latent form (10). The presence of an inhibitor in the affected corneal cells, similar to that demonstrated in the Burkitt's lymphoma cells, could be involved in maintenance of the viral genome in a latent state.

Clarification of the chemical nature of the inhibitor and its mechanism of action will require considerable purification of the crude

extracts used in these experiments. If the material can be highly purified and characterized, it is possible that it might have some therapeutic value despite the fact that it is prepared from cultures derived from neoplastic tissue. It is also possible that similar inhibitory activity might be found in extracts of lymphoid cell cultures derived from normal human lymphocytes.

Summary. Extracts of Burkitt's lymphoma cell cultures which had been previously shown to contain an inhibitor of herpes simplex virus *in vitro* were used to treat rabbits with ocular herpes infection. Topical instillation of the extract into the eyes of the rabbits cured or suppressed the progression of herpetic keratitis. Histopathologic studies of the eyes of treated and control animals confirmed the inhibition of keratitis and other evidence of herpes-related ocular inflammation.

We are grateful to Miss F. Y. Legallais, Mrs. S. A. Tyrrell and Mrs. Paula Miller for technical assistance.

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Received March 31, 1971. P.S.E.B.M., 1971, Vol. 138.